(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 October 2002 (24.10.2002)

PCT

(10) International Publication Number
WO 02/084294 A2

(51) International Patent Classification[7]: G01N 33/576, C12Q 1/28, C07K 16/00, A61P 31/00, A61K 39/00, C12N 15/00, A61K 48/00

(21) International Application Number: PCT/EP02/04167

(22) International Filing Date: 15 April 2002 (15.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/283,345 13 April 2001 (13.04.2001) US

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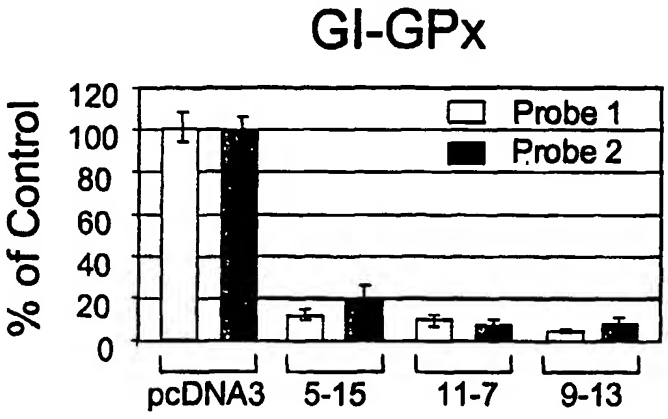
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: HUMAN CELLULAR PROTEIN GASTROINTESTINAL GLUTATHIONE PEROXIDASE AS TARGET FOR MEDICAL INTERVENTION AGAINST HEPATITIS C VIRUS INFECTIONS




(57) Abstract: The present invention relates to the human cellular protein glutathione peroxidase-gastrointestinal as potential targets for medical intervention against Hepatitis C virus (HCV) infections. Furthermore, the present invention relates to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections and a method for detecting Hepatitic C virus infections in an individual or in cells. Also mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production wherein said antibodies may be used.

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# Human cellular protein gastrointestinal glutathione peroxidase

# as target for medical intervention against Hepatitis C virus infections

## Specification

The present invention relates to the human cellular protein glutathione peroxidase-gastrointestinal as potential targets for medical intervention against Hepatitis C virus (HCV) infections. Furthermore, the present invention relates to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections and a method for detecting Hepatitis C virus infections in an individual or in cells. Also mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production wherein genes or said antibodies may be used.

### *Background of the invention*

Hepatitis C Virus (HCV) infection is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. The WHO estimates that approximately 3% of the world population, or 170 million people, have been infected with the Hepatitis C Virus. In the U.S., an estimated 3.9 million Americans have been infected (CDC fact sheet Sept. 2000). Over 80% of HCV-infected individuals develop chronic hepatitis, which is associated with disease states ranging from asymptomatic carrier states to repeated inflammation of the liver and serious chronic liver disease. Over the course of 20 years, more than 20% of chronic HCV-patients are expected to be at risk to develop cirrhosis or progress to hepatocellular carcinoma. Liver failure from chronic hepatitis C is the leading indicator for liver transplantation. Excluding transplantation, the CDC estimates that medical and work-loss cost for HCV annually are around $600 million. HCV is transmitted primarily by blood and blood products. Due to routine screening of the blood supplies from mid-1992, new transfusion-related cases are exceedingly rare and have been surpassed by injection drug use as the highest risk factor for acquiring the virus. There is also a sexual, however inefficient, route of transmission, and a 6% rate of transmission from infected mothers to their children, which is higher in case of HIV co-infection. In a certain percentage of infections, the mode of transmission remains unknown. In spite of the significant decline in incidence in

the 1990's, the number of deaths (estimated deaths annually at the moment: 8000 to 10,000 in U.S.) and severe disease due to HCV is anticipated to triple in the next 10 to 20 years. (Sources: CDC fact sheet (accessed 12/12/00); Houghton M. Hepatitis C Viruses. In BN Fields, DM Knipe, PM Howley (ed.) Fields Virology. 1996. Lippencott-Raven Pub., Philadelphia; Rosen HR and Gretch DR, Molecular Medicine Today Vol5, 393, Sept. 99; Science 285, 26, July 99: News Focus: The scientific challenge of Hepatitis C; Wong JB et al, Am J Public Health, 90, 1562, Oct 2000: Estimating future hepatitis C morbidity, mortality, and costs in the United States).

According to the announcement from the EASL (European Association for the Study of the Liver) International Consensus Conference on Hepatitis C (February 26-28, 1999, Paris, France), combination therapy of alpha interferon and ribavirin is the recommended treatment for naive patients. Monotherapy with interferon has also been approved by the FDA, but the sustained response rate (HCV RNA remains undetectable in the serum for more than 6 months after end of therapy) is only 15 to 20%, in contrast to 35 to 45% with combination therapy. Interferons (Intron A, Schering-Plough; Roferon A, Hoffmann-LaRoche; Wellferon, Glaxo Wellcome; Infergen, Amgen) are injected subcutaneously three times a week, ribavirin (Rebetol, Schering-Plough) is an oral drug given twice a day. Recommended treatment duration is 6 to 12 months, depending on HCV genotype. Experimental forms of slow-release pegylated interferons (Pegasys, Hoffmann-LaRoche; PEG-Intron, Schering-Plough) have shown improvements in response rates (42 to 82% in combination with ribavirin) and application (once-weekly injection) in recent clinical studies (Hepatology 32:4, Pt 2 of 2. Oct 2000; NEJM 343, 1673. Dec 2000; NEJM 343, 1666. Dec 2000). Common side effects of interferon therapy include: e.g. fatigue, muscle aches, head aches, nausea, fever, weight loss, irritability, depression, bone marrow suppression, reversible hair loss. The most common side effects of ribavirin are anemia, fatigue and irritability, itching, skin rash, nasal stuffiness, sinusitis, cough. More serious side effects of mono-and combination therapy occur in less than two percent of patients (NIDDK information: Chronic Hepatitis C: Current Disease Management. accessed 09.12.99). Some of the contraindications to interferon are psychosis or severe depression; neutropenia and/or thrombocytopenia; organ transplantation except liver; symptomatic heart disease; decompensated cirrhosis; uncontrolled seizures. Contraindications to ribavirin are end-stage renal failure; anemia; hemoglobinopathies; severe heart disease; pregnancy; no reliable method of contraception (consensus statement EASL).

Experimental treatments that are not new forms of interferon are Maxamine (histamine dihydrochloride, Maxim Pharmaceuticals), which will be combined with Interferon in phase III studies, VX-497 (Vertex Pharmaceuticals), an IMP dehydrogenase inhibitor, as a less toxic ribavirin substitute in phase II, and amantadine (Endo Labs), an approved influenza drug, as the third component in triple therapy (phase II). Inhibitors for HCV enzymes such as protease inhibitors, RNA polymerase inhibitors, helicase inhibitors as well as ribozymes and antisense RNAs are under preclinical development (Boehringer Ingelheim, Ribozyme Pharmaceuticals, Vertex Pharmaceuticals, Schering-Plough, Hoffmann-LaRoche, Immusol, Merck etc.). No vaccine is available for prevention or therapeutic use, but several companies are trying to develop conventional or DNA vaccines or immunostimulatory agents (e.g. Chiron, Merck/Vical, Epimmune, NABI, Innogenetics). In addition, antibodies against HCV virion have been developed and entered into clinical trials recently (Trimera Co., Israel).

In summary, the available treatment for chronic Hepatitis C is expensive, effective only in a certain percentage of patients and adverse side effects are not uncommon.

**Description of the invention**

Recent research has revealed how cells communicate with each other to coordinate the growth and maintenance of the multitude of tissues within the human body. A key element of this communication network is the transmission of a signal from the exterior of a cell to its nucleus, which results in the activation or suppression of specific genes. This process is called signal transduction.

Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class: receptors that constitute channels which, upon ligand interaction, allow signals to be passed in the form of small ion movement, either into or out of the cell. These ion movements result in changes in the electrical potential of the cells that, in turn, propagates the signal along the cell. More complex signal transduction involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and/or serine/threonine kinases. Protein phosphorylations change enzyme activities and protein

conformations. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cells.

Signal transducting receptors are of three general classes:

1. Receptors that penetrate the plasma membrane and have intrinsic enzymatic activity:

Receptors that have intrinsic enzymatic activities include those that are tyrosine kinases (e.g. PDGF, insulin, EGF and FGF receptors), tyrosine phosphatases (e.g. CD45 [*cluster determinant-45*] protein of T cells and macrophages), guanylate cyclases (e.g. natriuretic peptide receptors) and serine/threonine kinases (e.g. activin and TGF-beta receptors). Receptors with intrinsic tyrosine kinase activity are capable of autophosphorylation as well as phosphorylation of other substrates.

Additionally, several families of receptors lack intrinsic enzyme activity, yet are coupled to intracellular tyrosine kinases by direct protein-protein interactions. This class of receptors includes all of the cytokine receptors (e. g. the interleukin-2 receptor) as well as the CD4 and CD8 cell surface glycoproteins of T cells and the T cell antigen receptor.

2. Receptors that are coupled, inside the cell, to GTP-binding and hydrolyzing proteins (termed G-proteins):
Receptors of the class that interact with G-proteins all have a structure that is characterized by seven transmembrane spanning domains. These receptors are termed *serpentine* receptors. Examples of this class are the adrenergic receptors, odorant receptors, and certain hormone receptors (e.g. glucagon, angiotensin, vasopressin and bradykinin).

3. Receptors that are found intracellularly and upon ligand binding migrate to the nucleus where the ligand-receptor complex directly affects gene transcription:

The steroid/thyroid hormone receptor superfamily (e.g. glucocorticoid, vitamin D, retinoic acid and thyroid hormone receptors) is a class of proteins that reside in the cytoplasm and bind the lipophilic steroid/thyroid hormones. These hormones are capable of freely penetrating the hydrophobic plasma membrane. Upon binding ligand the hormone-receptor complex translocates to the nucleus and bind to specific DNA sequences resulting in altered transcription rates of the associated gene.

When the message reaches the nucleus via one or several of the pathways described above, it initiates the modulation of specific genes, resulting in the production of RNA and finally proteins that carry out a specific biological function. Disturbed activity of signal transduction molecules may lead to the malfunctioning of cells and disease processes. Specifically, interaction of HCV with host cells is necessary for the virus to replicate.

The present invention is based upon the surprising discovery that the human cellular protein glutathione peroxidase-gastrointestinal (P18283) is specifically downregulated as a result of HCV replication in HCV infected host cells. The antiviral therapeutic research approach described herein focuses on discovering the cellular signal transduction pathways involved in viral infections. Identification of the signal transduction molecules, key to viral infection, provides for, among other things, novel diagnostic methods, for example, assays and compositions useful therefore, novel targets for antiviral therapeutics, a novel class of antiviral therapeutics, and new screening methods (e.g. assays) and materials to discover new antiviral agents.

In order to develop new pharmaceutically active compounds, a potential target for medical intervention has to be identified. Thus, processes for finding pharmaceutically effective compounds include target identification.

Target identification is basically the identification of a particular biological component, namely a protein and its association with particular disease states or regulatory systems. A protein identified in a search for a pharmaceutically active chemical compound (drug) that can affect a disease or its symptoms is called a target. Said target is involved in the regulation or control of biological systems and its function can be interfered by with a drug.

The word disease is used herein to refer to an acquired condition or genetic condition. A disease can alter the normal biological system of the body, causing an over or under abundance of chemical compounds (chemical imbalance). The regulatory systems for these chemical compounds involve the use by the body of certain proteins to detect imbalances or cause the body to produce neutralizing compounds in an attempt to restore the chemical imbalance.

The word body is used herein to refer to any biological system, e.g. human, animal, cells, or cell culture.

It is object of the present invention to provide novel targets for medical intervention, prophylaxis and/or treatment of Hepatitis C virus infections in mammals, including humans, and cells or cell cultures together with methods for detecting HCV infections in individuals, cell cultures and cells and methods for detecting compounds useful for prophylaxis and/or treatment of HCV infections. The object of the present invention is solved by the teaching of the independent claims. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, and the examples of the present application.

**Detailed description of the invention**

It is now revealed for the first time that the human cellular protein glutathione peroxidase-gastrointestinal is specifically downregulated in a body as a result of HCV infection. This human cellular protein glutathione peroxidase-gastrointestinal has been identified as a novel diagnostic and therapeutic target for HCV infection.

Glutathione peroxidase:

Four distinct species of glutathione peroxidase have been identified in mammals to date, the classical cellular enzyme, the phospholipid hydroperoxide metabolizing enzyme, the gastroinestinal tract enzyme and the extracellular plasma enzyme. Their primary structures are poorly related. It has been shown that they are encoded by different genes and have different enzymatic properties. The physiological role of the human plasma enzyme remains still unclear due to the low levels of reduced glutathione in human plasma and the low reactivity of this enzyme.

The human cellular protein glutathione peroxidase-gastrointestinal (GI-GPx) is also known as glutathione peroxidase-related protein 2 (GPRP) or glutathione hydrogen peroxide oxidoreductase. It has been assigned to the Accession Number P18283 and the EC Number 1.11.1.9.

The human cellular protein glutathione peroxidase-gastrointestinal (GI-GPx) catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione (GSH) as hydrogen donor (2 GSH + $H_2O_2$ ⟶ GS—GS

+ 2 $H_2O$). It has a molecular weight of 84,000 and 4 subunits per mol of enzyme. The enzyme is useful for enzymatic determination of lipid hydroperoxide.

GI-GPx belongs to the family of selenoproteins and plays an important role in the defense mechanisms of mammals, birds and fish against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. It has been suggested that this enzyme functions in more times as a mechanism of protecting the cellular membrane system against peroxidative damage and that selenium as an essential trace element which may play an important role in this suggested function of the enzyme. It is known that both vitamin E and Se act as antioxidants also in a common mechanism of oxidative stress as an underlying cause of genetic changes.

Selenium functions within mammalian systems primarily in the form of selenoproteins. Selenoproteins contain selenium as selenocysteine and perform a variety of physiological roles. Seventeen selenoproteins have been identified: cellular or classical glutathione peroxidase; plasma (or extracellular) glutathione peroxidase; phospholipid hydroperoxide glutathione peroxidase; gastrointestinal glutathione peroxidase; selenoprotein *P*; types 1, 2, and 3 iodothyronine deiodinase; selenoprotein W; thioredoxin reductase; and selenophosphate synthetase. Of these, cellular and plasma glutathione peroxidase are the functional parameters used for the assessment of selenium status (D. H. Holben, A. M. Smith, *J. Am. Diet. Assoc.* 1999, 99, 836-843).

Beside vitamin E (DL-α-tocopherol), vitamin C (L-ascorbic acid), co-enzyme Q10, zinc, and selenium a lot of further antioxidants such as N-acetyl-L-cycteine, N-acetyl-S-farnesyl-L-cysteine, Bilirubin, caffeic acid, CAPE, catechin, ceruloplasmin, Coelenterazine, copper diisopropylsalicylate, deferoxamine mesylate, R-(-)-deprenyl, DMNQ, DTPA dianhydride, Ebselen, ellagic acid, (-)-epigallocatechin, L-ergothioneine, EUK-8, Ferritin, glutathione, glutathione monoethylester, α-lipoic acid, Luteolin, Manoalide, MCI-186, MnTBAP, MnTMPyP, morin hydrate, NCO-700, NDGA, p-Nitroblue, propyl gallate, Resveratrol, rutin, silymarin, L-stepholidine, taxifolin, tetrandrine, tocopherol acetate, tocotrienol, Trolox®, U-74389G, U-83836E, and uric acid (all available from Calbiochem, San Diego, CA, U.S.A.) which can be applied within the disclosed methods for preventing and/or treating HCV infections by compensating at least partially the down-regulation of GI-GPx.

Further antioxidants may be selected from the group of carboxylic acids such as citric acid and phenolic compounds such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallate, TBHQ (*tert*-butyl hydroquinone), tocopherols, lecithin, gums and resin guiac, THBP (trihydroxybutyrophenone), thiodipropionic acid and dilauryl thiodipropionate, and glycines.

Oxidative damage is mainly caused by free radicals, preferably reactive oxygen intermediates, derived from normal cellular respiration and oxidative burst produced when phagocytic cells destroy bacteria or virus-infected cells. In order to cope with the constant generation of potentially damaging oxygen radicals, eukaryotic organisms have evolved many defense mechanisms. These include the above-mentioned antioxidants which act as free radicals scavengers and which may interact with GI-GPx and/or may activate, stimulate, and/or increase the expression and/or production of GI-GPx. This advantageous effect of the radicals on the amount of GI-GPx generated in the cells competes with the HCV-induced down-regulation of GI-GPx and supports the cells in their fight against the Hepatits C viruses.

HCV infection studies:

The only reliable experimental HCV infection studies have been performed with chimpanzees. So far, there is no simple cell culture infection system available for HCV. Although a number of reports have been published describing *in vitro* propagation attempts of HCV in primary cells and cell lines, questions remain concerning reproducibility, low levels of expression and properly controlled detection methods (reviewed in J. Gen Virol. 81, 1631; Antiviral Chemistry and Chemotherapy 10, 99). Thus, the replicon system described by Bartenschlager and coworkers (Lohmann et al, Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110. 1999) was used for the studies disclosed herein. This replicon system reproduces a crucial part of the HCV replication cycle which is used as a system for simulating HCV infection. Bartenschlager's group produced bicistronic recombinant RNAs, so-called "replicons", which carry the neomycin-phosphotransferase (NPT) gene as well as a version of the HCV genome where the sequences for the structural HCV proteins were deleted. After transfection of the subgenomic HCV RNA molecules into the human hepatoma cell line HuH-7, cells supporting efficient RNA-dependent RNA replication of the HCV replicons were selected based on co-amplification of the NPT gene and resulting resistance to the antibiotic G-418. Integration of coding information into the cellular genome was an exclusion criteria

for functional replicons. Several lines were established from G-418 resistant clones with autonomously replicating HCV RNAs detectable by Northern Blotting. Minus-strand RNA replication intermediates were detected by Northern Blotting or metabolic radio-labeling, and the production of nonstructural HCV proteins was demonstrated by immuno-precipitation after metabolic labeling or Western Blotting.

Possible influences and/or dependencies of HCV's RNA-dependent RNA replication and nonstructural proteins on host cell transcription are accessible to analysis with the Clontech cDNA arrays used in the inventive methods described herein. HuH-pcDNA3 cells are HuH7 cells resistant to G-418 by integration of a NPT gene-carrying plasmid (pcDNA3, Invitrogen) and serve as negative control. Three replicon lines were analyzed for changes in cellular RNA expression patterns compared to the control line:

- HuH-9-13: cell line with persistant replicon I377/NS3-3'/wt, described in Science 1999, 285, 110-113,
- HuH-5-15: cell line with persistant replicon I389/NS3-3'/wt, described in Science 1999, 285, 110-113,
- HuH-11-7: cell line with persistant replicon I377/NS2-3'/wt, described in Science 1999, 285, 110-113.

These HCV replicon cells serve as a system for simulation of HCV infected cell systems, especially for simulating HCV infected mammals, including humans. Interference of HCV with the cellular signaling events is reflected in differential gene expression when compared to cellular signaling in control cells. Results from this novel signal transduction microarray analysis revealed significant downregulation of GI-GPx. Radioactively labeled complex cDNA-probes from HCV Replicon cells HuH-9-13, HuH-5-15, and HuH-11-7 were hybridized to cDNA-arrays and compared to hybridizations with cDNA-probes from HuH-pcDNA control cells which did not contain HCV Replicons.

Based on the surprising results reported herein, one aspect of the present invention is directed to a screening method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections. Specifically, this method involves contacting a test compound with GI-GPx and detecting the GI-GPx activity.

Another aspect of the present invention is directed to a diagnostic method, an assay for detecting Hepatitis C virus infections in an individual or in cells. This method involves providing a sample from the individual or providing cells and detecting activity of GI-GPx.

As sample could be used, for instance, blood, biopsies, cells or cell cultures of liver or of any other tissue infected with HCV.

Accordingly, one aspect of the present invention is directed to novel compounds useful in the above-identified methods. Therefore, the present invention relates to monoclonal or polyclonal antibodies that bind to GI-GPx.

Furthermore, the present invention discloses a method for treating Hepatitis C virus infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of GI-GPx or which inhibits at least partially the production of GI-GPx in the cells.

A similar aspect of the present invention is directed to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of GI-GPx or which inhibits at least partially the production of GI-GPx.

Another object of the present invention is to provide a method for regulating the production of Hepatitis C virus in an individual or in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of an agent wherein said agent inhibits at least partially the activity GI-GPx or wherein said agent at least partially inhibits the production GI-GPx in the cells . The above-mentioned monoclonal or polyclonal antibodies directed against GI-GPx may be used as pharmaceutically active agents within said methods.

In addition to the above-mentioned methods the present invention is also directed to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which activates at least partially GI-GPx or which activates or stimulates the production of GI-GPx in the cells.

Another inventive aspect is related to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of an agent which activates at least partially the activity of GI-GPx or which activates or stimulates at least partially the production of GI-GPx.

The term "associated diseases" refers to, for instance, opportunistic infections, liver cirrhosis, liver cancer, hepatocellular carcinoma, or any other diseases that can come along with HCV infection.

The function of GI-GPx is to detoxify peroxides in cells and prevent the cells from oxidative damage. As demonstrated in Fig. 3, subjecting HCV infected cells to oxidative stress conditions, preferably induced by paraquat or radicals generated from peroxides, leads to a decreased resistance of HCV infected cells in comparison to uninfected cells against toxicity of radicals. Thus, generating artificial oxidative stress conditions allows selective killing of HCV-infected cells.

Examples for useful radical forming compounds (radical initiators) are bipyridyls such as paraquat, 2,2'-bipyridyl and 4,4'-bipyridyl derivatives, bis-6-(2,2'-bipyridyl)-pyrimidines, tris-(2,2'-bipyridyl)-ruthenium, peroxides such as dibenzoylperoxid, diacetylperoxide, hydrogen peroxide, di-tert.-butylperoxide, or diaza compounds such as diazaisobutyronitril.

Yet another aspect of the present invention is directed to a novel therapeutic composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or associated diseases comprising at least one agent capable of inactivating or inhibiting the activity of GI-GPx or of decreasing or inhibiting the production and/or expression of GI-GPx.

Further embodiments of the present invention are represented by methods for regulating the production of Hepatitis C virus in an individual or in cells or cell cultures comprising the step of administering an individual or the cells a pharmaceutically effective amount of an agent wherein said agent activates or increases at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal.

Further aspects of the present invention relate to methods either for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual or in cells or cell culture comprising the step of administering either the individual or the cells or cell culture a pharmaceutically effective amount of an agent wherein said agent stimulates or increases at least partially the transcription of DNA and/or the translation of RNA encoding GI-GPx.

According to the above-mentioned method another aspect of the present invention is directed to novel therapeutic compositions useful within said methods for prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or associated diseases. Said compositions comprise at least one agent capable of increasing the activity of GI-GPx or of activating or stimulating the production and/or expression of GI-GPx.

Said pharmaceutical compositions may further comprise pharmaceutically acceptable carriers, excipients, and/or diluents.

As used herein, the term "inhibitor" refers to any compound capable of downregulating, decreasing, inactivating, suppressing or otherwise regulating the amount and/or activity of GI-GPx or its expression. Generally, GI-GPx inhibitors may be proteins, oligo- and polypeptides, nucleic acids, genes, small chemical molecules, or other chemical moieties.

Small chemical molecules are, for instance, organic compounds with molecular weight typically below 500 g/mol and preferably also with less than 10 heteroatoms.

As used herein, the term "activator" refers to any chemical compound capable of upregulating, activating, stimulating, or increasing the amount and/or activity of GI-GPx or its expression. Generally, said agents may be proteins, oligo- and polypeptides, nucleic acids, genes, small chemical molecules, or other chemical moieties. An example for an activator of glutathione peroxidase is e.g. selenium and retinoic acid (Brigelius-Flohé, R.,1999, Free Radicals in Biology and Medicine, 27, 951-965; Chu et al., 1999, Journal of Nutrition 129, 1846 – 1854).

The term "agent" is used herein as synonym for regulator, inhibitor, and/or activator. Thus, the term "agent" refers to any chemical or biological compound capable of down- or upregulating, de- or increasing, suppressing or stimulating,

inactivating or activating, or otherwise regulating or effecting the amount and/or activity of GI-GPx and/or the expression of GI-GPx.

One special kind of said agents are aptamers which function as regulators of the activity of a wide range of cellular molecules such as GI-GPx. Aptamers are nucleic acid molecules selected in vitro to bind small molecules, peptides, or proteins with high affinity and specificity. Aptamers not only exhibit highly specific molecular recognition properties but are also able to modulate the function of their cognate targets in a highly specific manner by agonistic or antagonistic mechanisms. Most famous examples for aptamers are DNA aptamers or RNA aptamers.
In addition to the role in transmitting genetic information from DNA to proteins, RNA molecules participate actively in many cell processes. Examples are found in translation (rRNA, tRNA, tmRNA), intracellular protein targeting (SRP), nuclear splicing of pre-mRNA (snRNPs), mRNA editing (gRNA), and X-chromosome inactivation (Xist RNA). Each of these RNA molecules acts as a functional product in its own right, without coding any protein. Because RNA molecules can fold into unique shapes with distinct structural features, some RNAs bind to specific proteins or small molecules (as in the ATP-binding aptamer), while others catalyze particular chemical reactions. Thus, RNA aptamers can be used to interact with GI-GPx and thereby modulate, regulate, activate, or inhibit the activity and biological function of said peroxidase.

As used herein, the term "regulating expression and/or activity" generally refers to any process that functions to control or modulate the quantity or activity (functionality) of a cellular component. Static regulation maintains expression and/or activity at some given level. Upregulation refers to a relative increase in expression and/or activity. Accordingly, downregulation refers to a relative decrease in expression and/or activity. Downregulation is synonymous with inhibition of a given cellular component's activity.

Further aspects of the present invention relate to methods either for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual or in cells or cell cultures comprising the step of administering either the individual or the cells or cell cultures a pharmaceutically effective amount of an agent wherein said agent inhibits or decreases at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

Therapeutics, pharmaceutically active agents or inhibitors, respectively, may be administered to cells from an individual *in vitro*, or may involve *in vivo* administration to the individual. The term "individual" preferably refers to mammals and most preferably to humans. Routes of administration of pharmaceutical preparations to an individual may include oral and parenteral, including dermal, intradermal, intragastral, intracutan, intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical or transdermal application, but are not limited the these ways of administration. For instance, the preferred preparations are in administratable form which is suitable for oral application. These administratable forms, for example, include pills, tablets, film tablets, coated tablets, capsules, powders and deposits. Administration to an individual may be in a single dose or in repeated administrations, and may be in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier, binder, lubricant, excipient, diluent and/or adjuvant. Pharmaceutically acceptable salt forms and standard pharmaceutical formulation techniques are well known to persons skilled in the art.

As used herein, a "pharmaceutical effective amount" of a GI-GPx activator or GI-GPx inhibitor is an amount effective to achieve the desired physiological result, either in cells or cell cultures treated *in vitro* or in a subject treated *in vivo*. Specifically, a pharmaceutically effective amount is an amount sufficient to inhibit, for some period of time, one or more of the clinically defined pathological processes associated with the viral infection. The effective amount may vary depending on the specific GI-GPx inhibitor selected, and is also dependent on a variety of factors and conditions related to the subject to be treated and the severity of the infection. For example, if the inhibitor is to be administered *in vivo*, factors such as the age, weight and health of the patient as well as dose response curves and toxicity data obtained in pre-clinical animal work would be among those considered. If the inhibitor is to be contacted with the cells or cell cultures *in vitro*, one would also design a variety of pre-clinical *in vitro* studies to assess such parameters as uptake, half-life, dose, toxicity, etc. The determination of a pharmaceutically effective amount for a given agent is well within the ability of those skilled in the art.

It is also apparent to a person skilled in the art that detection includes any method known in the art useful to indicate the presence, absence, or amount of a

detection target. Such methods may include, but are not limited to, any molecular or cellular techniques, used singularly or in combination, including, but not limited to: hybridization and/or binding techniques, including blotting techniques and immunoassays; labeling techniques (chemiluminescent, colorimetric, fluorescent, radioisotopic); spectroscopic techniques; separations technology, including precipitations, electrophoresis, chromatography, centrifugation, ultrafiltration, cell sorting; and enzymatic manipulations (e.g. digestion).

The present disclosure teaches for the first time the downregulation of GI-GPx specifically involved in the viral infection of Hepatitis C virus. Thus, the present invention is also directed to a method useful for detecting novel compounds useful for prophylaxis and/or treatment of HCV infections.

Methods of the present invention identify compounds useful for prophylaxis and/or treatment of HCV infections by screening a test compound, or a library of test compounds, for its ability to inhibit or activate GI-GPx, identified herein as characteristically downregulated during HCV growth and RNA replication inside a cell or individual. A variety of assay protocols and detection techniques are well known in the art and easily adapted for this purpose by a skilled practitioner. Such methods include, but are not limited to, high throughput assays (e.g., microarray technology, phage display technology), and *in vitro* and *in vivo* cellular and tissue assays.

In a related aspect, the present invention provides, in view of the important role of GI-GPx in the HCV infection and/or replication process, an assay component specially useful for detecting HCV in an individual, in cell cultures, or in cells. Preferably the assay component comprises oligonucleotides immobilized on a solid support capable of detecting GI-GPx activity. Preferably the solid support would contain oligonucleotides of sufficient quality and quantity to detect all of the above-mentioned human cellular proteins (e.g., a nucleic acid microarray).

Similarly, it is an object of the present invention to provide an assay component specially useful for screening compounds useful for the prophylaxis and/or treatment of HCV infections. One preferred assay component comprises oligonucleotides that encode GI-GPx immobilized on a solid support.

The polypeptide product of gene expression may be assayed to determine the amount of expression as well. Methods for assaying for a protein include, but are

not limited to, Western Blotting, immuno-precipitation, radioimmuno assay, immuno-histochemistry and peptide immobilization in an ordered array. It is understood, however, that any method for specifically and quantitatively measuring a specific protein or mRNA product can be used.

The present invention further incorporates by reference in their entirety techniques well known in the field of microarray construction and analysis. These techniques include, but are not limited to, techniques described in the following patents and patent applications describing array of biopolymeric compounds and methods for their fabrication:

U.S. Pat. Nos. 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,445,934; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,556,752; 5,561,071; 5,559,895; 5,624,711; 5,639,603; 5,658,734; 5,807,522; 6,087,102; WO 93/17126; WO 95/11995; WO 95/35505; EP 742 287; and EP 799 897.

Techniques also include, but are not limited to, techniques described in the following patents and patent application describing methods of using arrays in various applications:

U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,994,076; 6,033,860; 6,040,138; 6,040,140; WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280

It is readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are evident and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

## Description of Figures

Fig. 1:

Replicon cells express less GI-GPx mRNA than control HuH7 cells:

HuH7 control cells (pcDNA3) and the HuH replicon cell lines 5-15, 11-7 and 9-13 were plated in 10-cm culture dishes ($5x10^5$ cells/58 $cm^2$) and harvested after three days when cells were actively progressing through the cell cycle. Total RNA was isolated and 10 µg separated in a 1.2% agarose gel and used for Northern blot analysis.

**A:** Blots were hybridized with radioactively labeled oligonucleotides (Probe 1: open bar and Probe 2: filled bar) complementary to the mRNA coding for human gastrointestinal glutathione peroxidase (GI-GPx). Membrane was exposed to Kodak x-ray films for one day at –80°C with intensifier screens. The films were scanned and the density of the mRNA coding for GI-GPx calculated. The value for the control cells (pcDNA3) were set as 100% and compared with the values of the three replicon cell lines (± SEM), as indicated.

**B:** Blots were stripped and re-hybridized with two oligonucleotides (Probe 1: open bars and Probe 2: filled bars) recognizing the classic glutathione peroxidase (cGPx) mRNA. Membrane was exposed to Kodak x-ray films for two days at –80°C with intensifier screens. The film was densitrometrically scanned, the intensities of the cGP mRNA of the control cell line pcDNA3 (set as 100%) compared with the replicon cell lines, as indicated.

The data shown are the results of three independent experiments.

Fig. 2:

Cellular activity of glutathione peroxidase is reduced in replicon cell lines:

Cultures were plated and harvested as described in the experimental part below. 180 µg protein of cytosolic extract were used for estimation of glutathione peroxidase activity as described in Materials and Methods. The mean change (±SEM) of extinction at 340 nm reflecting glutathione peroxidase activity for each cell line is illustrated.

Fig. 3:

Replicon cells are susceptible towards oxidative stress:

Cells were plated in 96-well microtiter plates ($5x10^3$ cells/0.35$cm^2$) and after three days treated for 24 hours with the concentration of paraquat depicted. Cell viability was measured utilizing an Alamar-Blue assay and is reflected by relative

fluorescence units (RFU) at 405 nm. The $LD_{50}$ values (±SEM) of three independent experiments are shown for each cell line.

Fig. 4:

Effect of interferon on GI-GPx-, PKR- and genomic HCV-RNA levels:

The HuH7 pcDNA3 control cells and the replicon cell lines 5-15, 11-7 and 9-13 were plated as described in legend to Fig. 1 and after three days (Day 0) treated for two (Day 2) and four days (Day 4) with 1000U/ml interferon α (IFNα). Then, cultures were harvested and RNA was prepared. 10 µg of total RNA were used for Northern blot analysis. For detection of GI-GPx (A) Probe 1 was used (see Fig. 1). The membranes were stripped and successively hybridized with probes for PKR (B) and neomycin phosphotransferase (Neo)(C).

Exposure time for all blots were two days at –80°C with intensifier screen. The autoradiograms were densitrometrically scanned and the values compared with the maximal value obtained with each probe in the respective experiment. The values depicted (±SEM) are obtained from three independent experiments.

Fig. 5 and Fig. 6:

*Overexpression of GI-GPx in replicon cells causes downregulation of HCV:*

The replicon cell lines 5-15, 11-7 and 9-13 were plated at a density of $10^5$ cells per well of a 6-well plate and infected with $10^3$ Adenovirus particles/cell containing either the green fluorescent protein (GFP) as negative control, the GI-GPx cDNA without the 3'UTR (- UTR) and with the 3'UTR containing the SECIS (+ UTR), as indicated.

After four (d4) and seven days (d7) post infection cultures were harvested and 10 µg protein separated on a 12.5 % polyacrylamide gel. Western blot analysis was performed using an NS5a antibody (Fig. 5). Expression of the transduced GI-GPx cDNA was monitored with a GI-GPx-specific antiserum (Fig. 6).

The x-ray films were densitometrically scanned and the NS5a values compared with untransfected control cells (set as 100 %) (Fig. 5) and the GI-GPx values compared with the maximum expression of the transduced GI-GPx cDNA obtained seven days post infection (set as 100%) (Fig. 6).

A considerable over-expression of the GI-GPx protein was observed, when cells were infected with the GI-GPx +3'UTR virus and slight overexpression of GI-GPx was observed with the GI-GPx -3'UTR virus (Fig. 6).

The data show a drastic down-regulation of the HCV protein NS5a in all replicon cell lines infected with the GI-GPx+3'UTR virus (Fig. 5).

Loading efficiency and integrity of proteins was controlled with a tubulin antibody (data no shown). The values depicted (±SEM) are obtained from three independent experiments.

Examples

1. Human cDNA-arrays on membranes

Atlas™ Human Stress Array (Catalog #: 7747-1) from Clontech (Clontech Laboratories, Palo Alto, CA 94303-4230, USA) were used. This array includes 234 human cDNAs immobilized in duplicate dots (10 ng of cDNA per dot) on a nylon membrane.

2. Cellular HCV RNA replication system

HuH-pcDNA3, HuH-9-13, HuH-5-15 and HuH-11-7 cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FCS (fetal calf serum), 2 mM Glutamine, Penicillin (100 IU/ml) / Streptomycin (100 µg/ml) and 1x nonessential amino acids in the presence of 1 mg/ml G-418. Cells were routinely passaged three times a week at a dilution of 1:3 or 1:2.

3. Lysis of cells, and isolation of total RNA

HuH-pcDNA3, HuH-9-13, HuH-5-15 and HuH-11-7 cells were seeded at 5 x $10^5$ cells per 10 cm plate in medium without G-148. The medium was changed 3 days after plating and cells were harvested 5 days after plating by lyzing the cells directly on the plate with 4 ml of Tri-reagent (Molecular Research Center, Inc., USA). The lysates were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 15 minutes at 4°C. The supernatant was mixed with 0,1 ml of 1-bromo-3-chloropropane per 1 ml of Tri reagent and vigorously shaken. The suspension was stored for 5 minutes at room temperature and then centrifuged at 12000xg for 15 minutes at 4°C. The colorless upper phase was transferred into new tubes, mixed with 5 µl of polyacryl-carrier (Molecular Research Center Inc., USA) and with 0.5 ml of isopropanol per 1 ml of Tri reagent and vigorously shaken. The samples were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 8 minutes at 4°C. The supernatant was removed and the RNA pellet washed twice with 1 ml of 75% ethanol. The pellet was dried and resuspended in 25 µl of RNase-free buffer per initial 1 ml lysate.

4. Preparation of radioactively labeled cDNA probes from RNA

In order to obtain radioactively labeled cDNA probe, RNA was transcribed into a cDNA-probe in the presence of radioactively labeled dATP. 6 µg of total RNA

was labeled with 100 µCi [$^{33}$P]-dATP (Amersham, UK) according to the protocol provided by Clontech. Subsequently, the reaction was stopped by adding 5 µl 0.5M EDTA (ethylene diamine tetraacetate) and 25 µl 0.6M NaOH and incubation for 30 minutes at 68°C.

Unincorporated nucleotides were removed from the labeling reaction using ProbeQuant G-50 columns (Amersham, UK). The column was vigorously shaken and centrifuged for 1 minute at 735x*g* in an appropriate reaction tube after bottom closure and lid were removed. The column was placed into a new reaction tube, the probe was applied onto the center of a column material and the column was centrifuged for 2 minutes at 735x*g*. The flow-trough was transferred into new reaction tubes and filled up to a volume of 100 µl with 10 mM Tris, pH 7.4, 1 mM EDTA. The probe was precipitated by centrifugation for 15 minutes at 12000x*g* after 4 µl of 5M NaCl, 1 µl poly-acryl-carrier (Molecular Research Centre, Inc., USA) and 250 µl ethanol were added. The supernatant was discarded and the pellet dried before starting with the hybridisation.

5. Hybridisation of radioactively labeled cDNA-probes to cDNA-arrays

The pellet was resuspended in 10 µl $C_0t$-1 DNA (1 µg/µl, Roche Diagnostics, Germany), 10 µl yeast tRNA (1 µg/µl Sigma, USA) and 10 µl polyA (1 µg/µl, Roche Diagnostics, Germany). Herring sperm DNA was added to a final concentration of 100 µg/ml and the volume was filled up to 100 µl with 5 µl 10% SDS (Sodiumdodecylsulfat), 25 µl 20x SSC (3 M NaCl, 300 mM Sodium Citrate, pH 7.0) and bidestilled $H_2O$. The mix was put on 95°C for 5 minutes, centrifuged for 30 seconds at 10000xg and vigorously shaken for 60 minutes at 65°C. A 1 µl aliquot of the probe was used to measure the incorporation of radioactive dATP with a scintillation counter. Probes with at least a total of $20x10^6$ cpm were used. The arrays were prehybridised for at least 3 hours at 65°C in hybridisation solution in a roller bottle oven. After prehybridisation the radioactively labeled probe was added into the hybridisation solution and hybridisation was continued for 20 hours. The probe was discarded and replaced with wash solution A (2xSSC). The arrays were washed twice in wash solution A at room temperature in the roller oven. Afterwards, wash solution A was replaced by wash solution B (2x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. Then, wash solution B was replaced by wash solution C (0.5x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. The moist arrays were wrapped in airtight bags and exposed for 8 to 72 hours on erased phospho-imager screens (Fujifilm, Japan).

6. Analysis of cDNA-arrays

The exposed phospho-imager screens were scanned with a resolution of 100 µm and 16bits per pixel using a BAS-1800 (Fujifilm, Japan). Files were imported into the computer program ArrayVision (Imaging Research, Canada). Using the program's features, the hybridisation signals of each target cDNA were converted into numbers. The strength of the hybridisation signals reflected the quantity of RNA molecules present in the probe. Differentially expressed genes were selected according to the ratio of their signal strength after normalization to the overall intensity of the arrays.

7. Results

Comparing the expression pattern of signal transduction mRNAs in HCV Replicon cells HuH-9-13, HuH-5-15, and HuH-11-7 and HuH-pcDNA control cells which do not contain HCV Replicons using cDNA-arrays on membranes, the human gastrointestinal glutathione peroxidase (P18283) gene was identified as anti-HCV target. The mRNA levels were down-regulated to 2.8% in HuH-9-13, to 8.3% in HuH-5-15 and to 6.1% in HuH-11-7 cells compared to non-infected HuH-pcDNA control cells.

8. Northern Blotting

10 µg total RNA of each cell line was separated in a 1.2% agarose-formaldehyde gel, transferred on nylon membrane (Amersham) and hybridized with two different oligo-desoxyribonucleotides. Their sequences were derived from the coding (5'-TGG TTG GGA AGG TGC GGC TGT AGC GTC GGA AGG GC-3') and 3'-untranslated (5'-CCT CTC AGA CAC CAC CCA TGA GGG TTT AGG AAG GTG CCA T-3') region of the human gastrointestinal glutathione peroxidase (P18283) gene. Labeling was performed by 3'-end tailing with $^{32}$P-dCTP and terminal transferase (Roche Diagnostics GmbH, Mannheim, Germany). Northern Blotting membranes were hybridized with the labeled oligonucleotides over night at 65°C and unspecifically bound probe washed away.

After final washing (1x SSC, 1% SDS at 60°C for 30 min.) bound probe was detected by autoradiography for 12 hrs at –70°C using an x-ray film (Fuji) and quantified with a phospho-imager.

9. Confirmation of expression pattern by Nothern Blotting

Northern blot analysis was performed with two oligonucleotide probes derived form the human gastrointestinal glutathione peroxidase cDNA. Hybridization resulted in detection of one RNA of about 1 kb in HuH-pcDNA3 cells, but no or only weak detection of this band in HCV-replicon carrying cells. Therefore, analysis of the Northern Blotting signals confirmed precisely the down-regulation previously observed in the filter array hybridization (cf. 6.; Fig. 1).

10. Glutathione Peroxidase Activity is reduced in Replicon cell lines

Measuring the glutathione peroxidase activity utilizing *tert* butyl hydroperoxide as substrate, revealed that the replicon cells comprise reduced glutathione peroxidase activity (Fig. 2).

Method:

For measuring cellular glutathione peroxidase activity, the description of the kit's manufacturer (Calbiochem) was followed. Briefly, cells were washed with ice-cold PBS (phosphate buffered saline), harvested with a rubber policeman in 5 mM EDTA, 1 mM DTT (dithiothreitol) and 50 mM Tris-HCl (Tris-(hydroxymethyl)-aminomethane-hydrochloride), pH 7.5 and lysed by three cycles of freezing and thawing. After spinning for 15 min at 10.000x*g* (4°C) protein concentration of the supernatant was determined with the BCA reagents (Pierce, Bruchsal, Germany). 180 µg protein were used per assay.

*Tert*-butyl hydroperoxide was used as substrate and GI-GPx activity was estimated indirectly. Oxidized glutathione, produced upon reduction of the peroxide by GI-GPx, is recycled to its reduced state by glutathione reductase by oxidation of $NADPH+H^+$ to $NADP^+$. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm ($A_{340}$), which provides a spectrophotometric means of monitoring GI-GPx activity. Thus, the rate of decrease in the $A_{340}$ (delta E in Fig. 2) is directly proportional to the GI-GPx activity in the sample.

11. Sensitivity of Replicon Cells towards Paraquat

Treating mock transfected and replicon cells with increasing amounts of paraquat, a compound which produces radicals intracellularly, showed enhanced susceptibility of replicon cells against this drug.

Paraquat impaired the viability of replicon cells more severely than of pcDNA3 control cells (Fig. 2). The estimated $LD_{50}$ values for paraquat calculated from three independent experiments were 260 ± 50µM for HuH 9-13, 270 ± 75µM for HuH 5-15, 310 ± 65µM for HuH 11-7 and 1250 ± 120µM for HuH pcDNA3 (cf. Fig. 3).

Methods:

Replicon cell lines and control cells were incubated for 24 hours with various concentrations of paraquat (methylviologen) and viability of the cultures were measured using the Alamar Blue assay.

For quantification of the degree of cell death in cell culture we employed the viability assay based on the reduction of tetrazolium salt to formazan by mitochondrial dehydrogenase activity. The assay was performed in 96-well microtiter plates (Greiner, Frickenhausen, Germany) as described previously (T. Herget et al., J. Neurochem. 1998, 70, 47–58) but Alamar Blue (Roche Molecular Biochemicals, Germany) was used instead of MTT. The light absorbance at 405 nm of the medium including all factors but without cells was determined and subtracted from the absorption readings with cells. Eight wells per sample point were analyzed and each experiment was repeated independently at least three times.

12. Inverse Regulation of HCV Replication and GI-GPx Expression

Replicon cells were incubated with interferon α (IFN) for two and four days. Northern blot analyses were performed with 10 µg total RNA. The IFN treatment (1000 U/ml) caused a time- and dose-dependent down-regulation of the HCV-replicon RNA and the HCV protein NS5a. An inverse correlated expression was observed for GI-GPx, i.e. GI-GPx was up-regulated within four days of interferon treatment. Interferon had no effect on the expression of GI-GPx in mock transfected HuH7 cells (cf. Fig. 4).

13. Ectopic Expression of GI-GPx in Replicon Cell Lines

The cDNA coding for the GI-GPx was cloned by RT-PCR from HuH7 cells. Transient expression of the GI-GPx protein in HEK293 cells caused an increase of glutathione peroxidase activity demonstrating its functionality. The construct was recombined into the genome of Adeno virus. Adeno virus carrying the GI-GPx cDNA was produced and used for transduction of the GI-GPx cDNA into HuH7 and replicon cells. Western blot analyses performed 4 and 7 days after

transfection showed a drastic down-regulation of the HCV protein NS5a. Such a down-regulation was not observed when 'empty' or the GFP (green fluorescent protein) gene-containing Adeno virus was used in parallel (cf. Fig. 5 and 6).

Methods:

The adenovirus used here were all E1, E3 defective derivatives of adenovirus type 5 (W.C. Russell, J. Gen. Virol. 2000, 81, 2573-2604). The coding region for GI-GPx (0.7 kb) was amplified by PCR using an upstream primer containing an *Hin*dIII recognition site (5'-GCG CAA GCT T*AT* GGC TTT CAT TGC CAA GTC CTT C-3', start codon in italic) and a downstream primer containing an XbaI site (5'-GTT CAT CTA GAT ATG GCA ACT TTA AGG AGG CGC TTG-3') but without stop-codon to allow expression of fusion proteins with HIS- and VSV-tag. The 3'-UTR (0.3 kb) of the GI-GPx mRNA, containing a SECIS (selenocysteine inserting sequence), was amplified using the up-stream primer 5'-GCC CTC GAG ATG TGA ACT GCT CAA CAC ACA G-3' with an XhoI recognition site and the down-stream primer 5'-CCA CGC GGC CGC TTT ATT GGT CTC TTC TAG CAG AGT GGC-3' covering the polyadenylation site (AAUAAA) and containing a NotI restriction site for cloning. RNA isolated from HuH7 cells were reverse transcribed and used as template for PCR. The cDNA coding for human GI-GPx was cloned into the transfer plasmid (pPM7) between the CMV (cytomegalo virus) immediately early promoter/enhancer and the rabbit beta-globin intron/polyadenylation signal. This expression cassette was inserted into a bacterial plasmid borne-adenovirus genome using recombination in bacteria (C. Chartier et al., J. Virol. 1996, 70, 4805-4810). A cloned version of the novel genome was identified, the viral genome was released from the plasmid by restriction enzyme digestion and virus replication was initiated by transfecting the genome into HEK 293 cells using a modified PEI transfection method (A.-I. Michou et al., J. Virol. 1999, 73, 1399-1410). Virus was amplified in modified HEK 293 cells (F.L. Graham et al., J. Gen. Virol. 1977, 36, 59-74) and purified from cell lysates using CsCl density gradient centrifugation as described (M. Cotten et al., Adenovirus polylysine DNA conjugates. In Current Protocols in Human Genetics; John Wiley and Sons, Inc. New York. 1996 pp. 12.3.1-12.3.33). Virus was quantified by protein content using the conversion factor 1 mg/ml pure virion protein = $3.4 \times 10^{12}$ viral particles/ml (P. Lemay et al., Virology 1980, 101, 131-143). The control viruses AdJ5 and AdLuc were previously described (J.B. Glotzer et al., J. Virol. 2001, 75, 2421-2434; J.B. Glotzer et al., Nature 2000, 407, 207-211).

## Claims

1. Method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections comprising:
   a) contacting a test compound with the human cellular protein glutathione peroxidase-gastrointestinal; and
   b) detecting said human cellular protein glutathione peroxidase-gastrointestinal activity.

2. Method for detecting Hepatitis C virus infections in an individual comprising:
   a) providing a sample from said individual; and
   b) detecting activity in said sample of human cellular protein glutathione peroxidase-gastrointestinal.

3. Method for detecting Hepatitis C virus infections in cells, cell cultures, or cell lysates comprising:
   a) providing said cells, cell cultures, or cell lysates; and
   b) detecting activity in said cells, cell cultures, or cell lysates of human cellular protein glutathione peroxidase-gastrointestinal.

4. An monoclonal or polyclonal antibody that binds to said human cellular protein glutathione peroxidase-gastrointestinal.

5. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which inhibits at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

6. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which inhibits at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

7. Method for regulating the production of Hepatitis C virus in an individual comprising the step of administering an individual a pharmaceutically effective amount of an agent wherein said agent inhibits at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially inhibits the production of said human cellular protein glutathione peroxidase-gastrointestinal.

8. Method for regulating the production of Hepatitis C virus in cells or cell culture comprising the step of administering a pharmaceutically effective amount of an agent wherein said agent inhibits at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially inhibits the production of said human cellular protein glutathione peroxidase-gastrointestinal in the cells or cell culture.

9. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which activates or stimulates at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

10. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of an agent which activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which activates or stimulates at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

11. Method for regulating the production of Hepatitis C virus in an individual comprising the step of administering an individual a pharmaceutically effective amount of an agent wherein said agent activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal.

12. Method for regulating the production of Hepatitis C virus in cells or cell culture comprising the step of administering a pharmaceutically effective amount of an agent wherein said agent activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal in the cells or cell culture.

13. Method according to any one of claims 5 – 8 wherein the agent is a monoclonal or polyclonal antibody which binds to said human cellular protein glutathione peroxidase-gastrointestinal.

14. Oligonucleotides that bind to the DNA or RNA encoding the human cellular protein glutathione peroxidase-gastrointestinal.

15. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent wherein said agent inhibits at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

16. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in cells or cell culture comprising the step of administering the cells or cell culture a pharmaceutically effective amount of an agent wherein said agent inhibits at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

17. Method according to any one of claims 5 – 8, 15, or 16 wherein said agent is an oligonucleotide which binds to the DNA and/or RNA encoding the human cellular protein glutathione peroxidase-gastrointestinal.

18. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent wherein said agent activates at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

19. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in cells or cell culture comprising the step of administering the cells or cell culture a pharmaceutically effective amount of an agent wherein said agent activates at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

20. Method for regulating the activity of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent wherein said agent interacts with said human cellular protein glutathione peroxidase-gastrointestinal.

21. Method for regulating the activity of the human cellular protein glutathione peroxidase-gastrointestinal in cells or cell culture comprising the step of administering the cells or cell culture a pharmaceutically effective amount of an agent wherein said agent interacts with said human cellular protein glutathione peroxidase-gastrointestinal.

22. Method for selective killing of HCV infected cells in an individual comprising the step of administering the individual a pharmaceutically effective amount of a radical initiator which is capable of generating artificial oxidative stress conditions within the cells.

23. Method for selective killing of HCV infected cells comprising the step of administering the cells or cell culture a pharmaceutically effective amount of a radical initiator which is capable of generating artificial oxidative stress conditions.

24. Method for preventing and/or treating HCV infections in an individual by at least partially compensating the down-regulation of GI-GPx comprising the step of administering to the individual a pharmaceutically effective amount of at least one antioxidant which is capable of supporting the function of GI-GPx present within the cells.

25. Method for preventing and/or treating HCV infections in cells by at least partially compensating the down-regulation of GI-GPx comprising the step of

administering to the cells or cell culture a pharmaceutically effective amount of at least one antioxidant which is capable of supporting the function of GI-GPx present within the cells.

26. Method according to any one of claims 5 – 21 wherein the agent is selected from the group comprising small chemical molecules which are organic compounds having a molecular weight below 500 g/mol, interferons, aptamers, antioxidants, and radical initiators.

27. Composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or diseases associated with HCV infection, said composition comprising at least one agent as defined in claim 13, 17, or 26 capable of inhibiting activity of said human cellular protein glutathione peroxidase-gastrointestinal or capable of decreasing the expression of said human cellular protein glutathione peroxidase-gastrointestinal.

28. Composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or diseases associated with HCV infection, said composition comprising at least one agent as defined in claim 13, 17, or 26 capable of increasing the activity of said human cellular protein glutathione peroxidase-gastrointestinal or capable of activating or stimulating the expression of said human cellular protein glutathione peroxidase-gastrointestinal.

29. Composition according to claim 27 or 28 further comprising pharmaceutically acceptable carriers, excipients, and/or diluents.

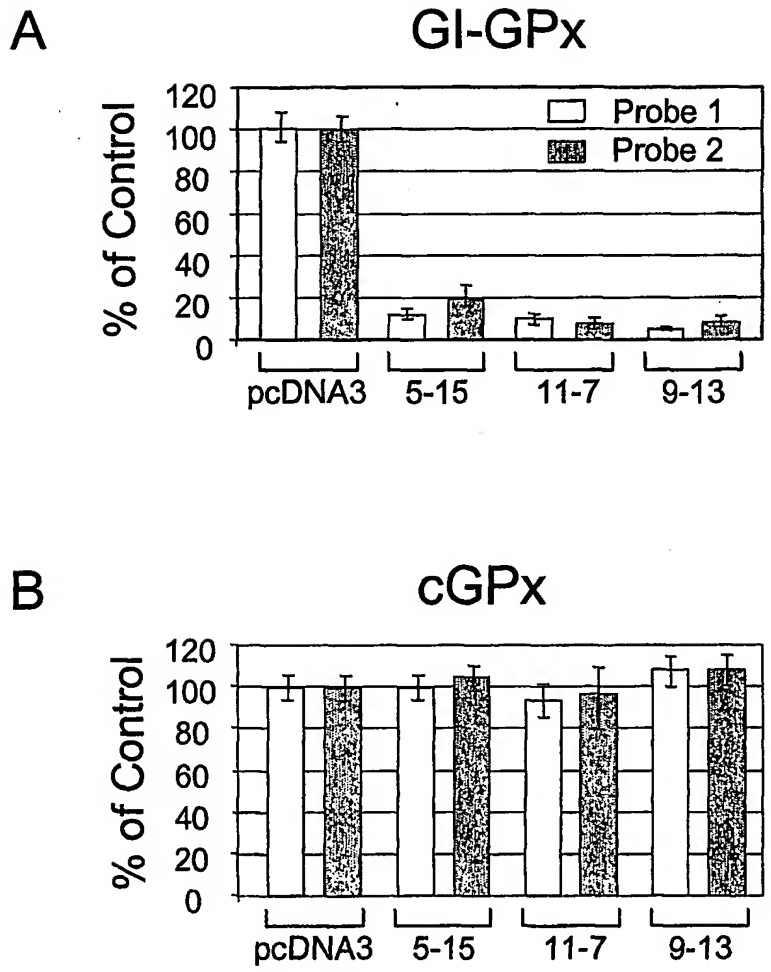
A
Gl-GPx
% of Control
120
100
80
60
40
20
0
Probe 1
Probe 2
pcDNA3
5-15
11-7
9-13
B
cGPx
% of Control
120
100
80
60
40
20
0
pcDNA3
5-15
11-7
9-13

Fig. 1

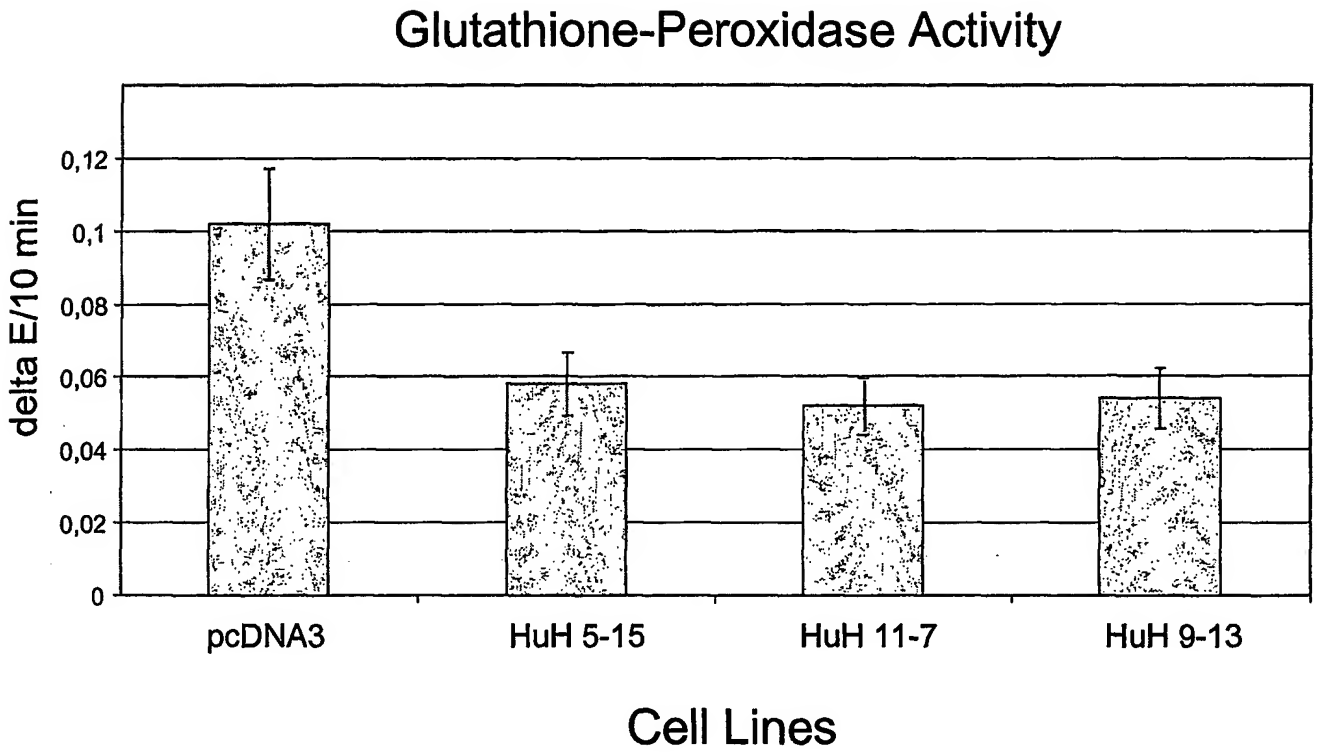
Glutathione-Peroxidase Activity
delta E/10 min
0,12
0,1
0,08
0,06
0,04
0,02
0
pcDNA3
HuH 5-15
HuH 11-7
HuH 9-13
Cell Lines

Fig. 2

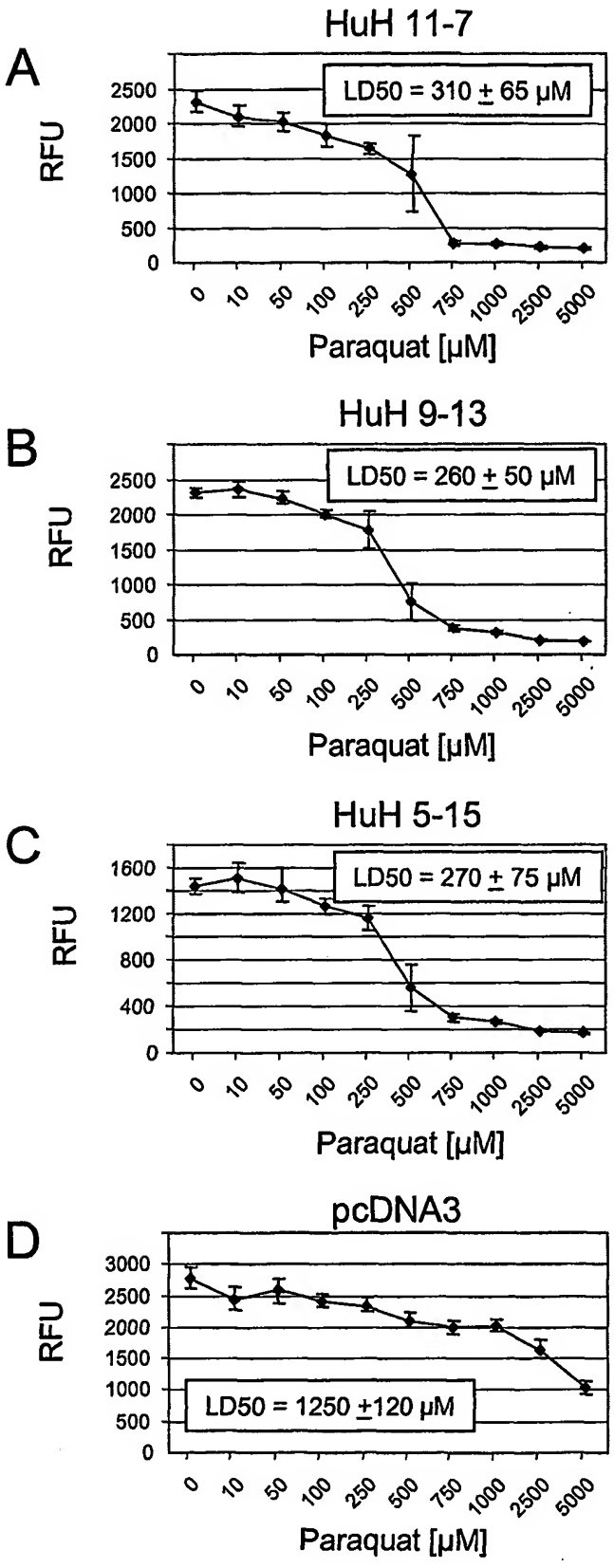
A
HuH 11-7
LD50 = 310 ± 65 µM
RFU
2500
2000
1500
1000
500
0
0 10 50 100 250 500 750 1000 2500 5000
Paraquat [µM]
B
HuH 9-13
LD50 = 260 ± 50 µM
RFU
2500
2000
1500
1000
500
0
0 10 50 100 250 500 750 1000 2500 5000
Paraquat [µM]
C
HuH 5-15
LD50 = 270 ± 75 µM
RFU
1600
1200
800
400
0
0 10 50 100 250 500 750 1000 2500 5000
Paraquat [µM]
D
pcDNA3
LD50 = 1250 ±120 µM
RFU
3000
2500
2000
1500
1000
500
0
0 10 50 100 250 500 750 1000 2500 5000
Paraquat [µM]

Fig. 3

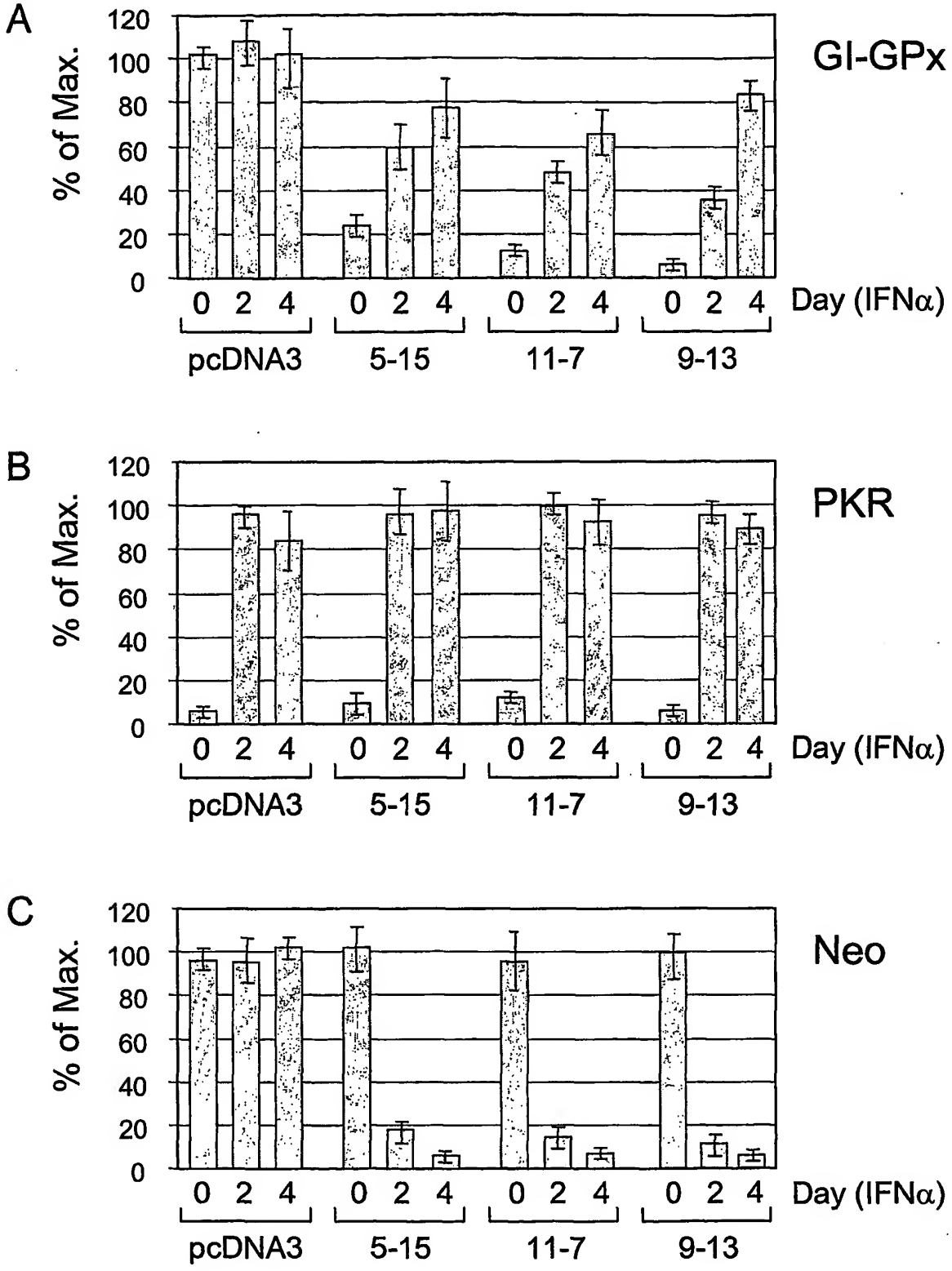
A
% of Max.
120
100
80
60
40
20
0
GI-GPx
0 2 4 0 2 4 0 2 4 0 2 4
Day (IFNα)
pcDNA3
5-15
11-7
9-13
B
% of Max.
PKR
Day (IFNα)
pcDNA3
5-15
11-7
9-13
C
% of Max.
Neo
Day (IFNα)
pcDNA3
5-15
11-7
9-13

Fig. 4

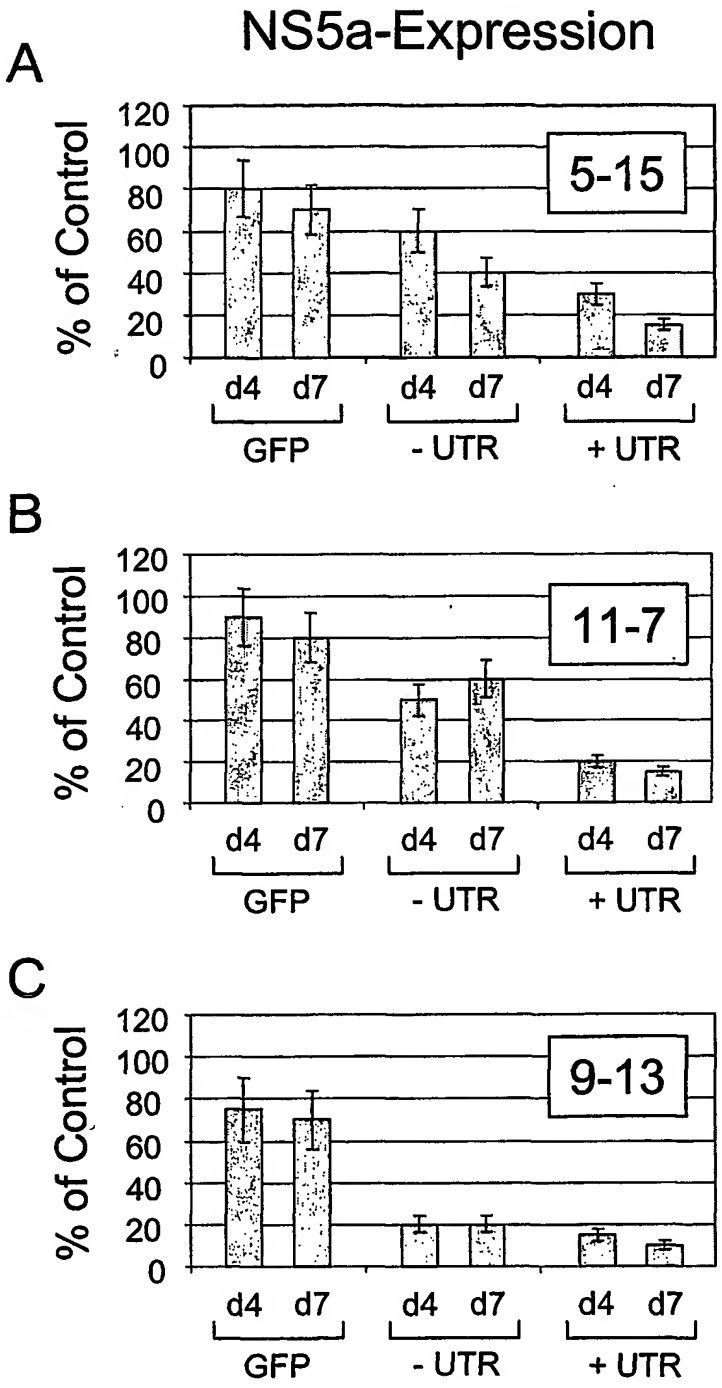
NS5a-Expression
A
% of Control
120
100
80
60
40
20
0
5-15
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR
B
% of Control
120
100
80
60
40
20
0
11-7
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR
C
% of Control
120
100
80
60
40
20
0
9-13
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR

Fig. 5

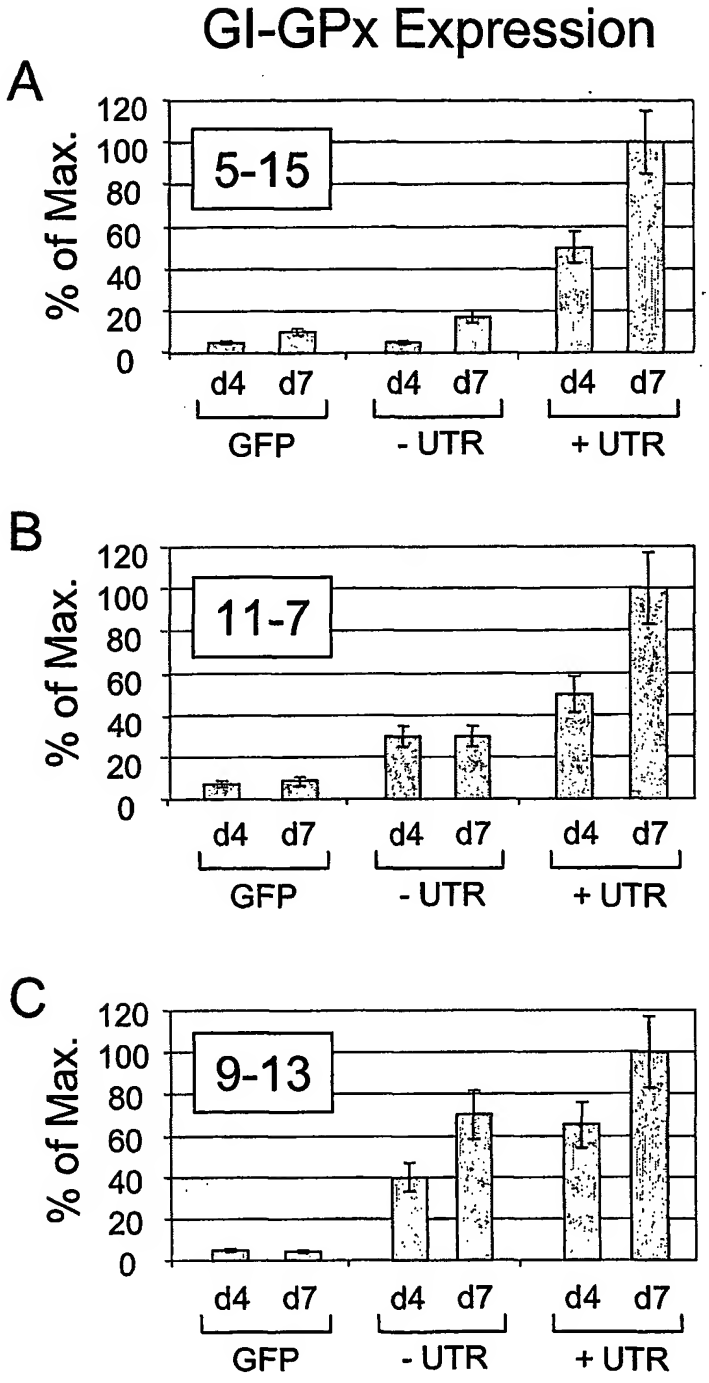
GI-GPx Expression
A
% of Max.
120
100
80
60
40
20
0
5-15
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR
B
% of Max.
120
100
80
60
40
20
0
11-7
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR
C
% of Max.
120
100
80
60
40
20
0
9-13
d4 d7 d4 d7 d4 d7
GFP - UTR + UTR

Fig. 6